Light and Scanning Electron Microscopic Studies of Spermatozoa from Infertile Couples Before and After Swim Up Migration

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Abstrak

Sebanyak 24 sampel semen pria pasangan infertil telah dievaluasi dengan mikroskop cahaya dan elektron skening sebelum dan sesudah spermatozoa dilakukan prosedur migrasi "swim up". Untuk menilai morfologi spermatozoa pada mikroskop cahaya digunakan pewarnaan "toluidine blue", sedangkan pada mikroskop elektron skening digunakan penyelubungan dengan karbon dan emas. Dalam membandingkan hasil sebelum dan sesudah spermatozoa dilakukan prosedur migrasi swim up, tampak bahwa rata-rata motilitas meningkat dari 61,5% menjadi 90,6%, kecepatan dari 44,4 menjadi 52,4 mikrometer per detik, dan jumlah persentase spermatozoa normal dari 48,5% menjadi 60,9%. Sebaliknya persentase spermatozoa abnormal menurun dari 51,5% menjadi 39,1%. Di antara spermatozoa abnormal, bentuk kepala kecil paling dominan dan jumlahnya lebih dari 10%. Nyata sekali bahwa dengan mikroskop elektron skening, keabnormalan kepala spermatozoa dapat lebih terdeteksi kendati dengan mikroskop cahaya tampak normal.

Abstract

Twenty-four samples of semen from male partners of infertile couples were evaluated before and after swim up migration by means of light and scanning electronic microscopy. For the evaluation of the spermatozoa morphology, toluidine blue staining was used for light microscopy, whereas carbon and gold coating was used for scanning electron microscopy. Comparison of results before and after the swim up migration showed that average motility increased from 61.5% to 90.6%, velocity from 44.4 to 52.4 micrometers per second, and the percentage of normal spermatozoa from 48.5% to 60.9%. The percentage of abnormal spermatozoa decreased from 51.5% to 39.1%. Among the abnormalities observed was a predominance of microcephalic sperm, which amounted to more than 10%. It was evident that under scanning electron microscopy, abnormalities of the sperm head could be more easily detected, that would not otherwise be detected under light micrsocopy.

Keywords : Swim up migration, Scanning electron microscopy, Infertile couples

INTRODUCTION

In many laboratories, the standards for evaluation of semen quality include several parameters, such as the concentration, motility, and velocity of the spermatozoa. One parameter which is often neglected or considered of lesser importance is sperm morphology. Under light microscopy, the evaluation of this aspect is based only on the shape of the head, oval being considered normal, while a non-oval shape (NOS) is regarded as abnormal.[1] As yet there is no agreement on the NOS threshold. Studies have proposed that NOS should not exeed 20%,[2] 40% [1,3] and 50%.[4] Eliasson states that sperm morphology is an important parameter, although there is no uniformity on the definition of "normal spermatozoa".[1]

Standard semen analysis covering sperm concentration, motility, and morphology provides some indication of spermatozoal function, or fertilization capacity.[5] Of the three parameters, motility and morphology show a better correlation with fertilization capacity[1], while Kruger *et al.*[6] confirm that only sperms with normal morphology show a significant correlation with fertilization. The examination of sperm morphology can give information on the condition of the testis seminiferous tubules.[7] MacLeod and Gold in 1951 have already detected a morphological difference between the spermatozoa of fertile and infertile males.[8]

Jeulin *et al.* reported that only normally shaped spermatozoa were able to penetrate the cervical mucus

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to reach the ovum in the Fallopian tube.[9] In spite of the fact that only normally shaped spermatozoa are able to reach the ovum, deformities occur in 3-4% of all neonates, while the abortion rate is 15%.[10] This indicates that normal sperm morphology does not guarantee the normality of the DNA, genes, or chromosomes in the sperm heads. Even allowing for the fact that such birth defects and miscarriages may also be due to the quality of the ovum fertilized or other factors as well.[10]

Although scanning electron microscopy fails to reveal the sperm head content, however slight deformities in the sperm head detected by this method to some extent reflect internal defects.[11] Success in the hamster zona-free oocyte/human sperm penetration assay (SPA) is related to a high percentage of normally shaped spermatozoa.[12] Nevertheless, the fertilizing ability of the spermatozoon concerned must remain in doubt, for this can be determined only by proving its capacity to penetrate the *zona pellucida* of the human ovum.[12]

Light microscopy spermatozoal morphology evaluation at a magnification of 1000 x can sufficiently distinguish oval shaped from NOS sperms. At a magnification of 10,000 x or more such as under the scanning electron microscope, oval spermatozoa diagnosed as normal under the light microscope sometimes showed abnormal features not visible under the light microscope.[1,11] The purpose of this investigation is to observe the quality of spermatozoa after the swim up migration procedure of the semen, and subsequently evaluate the 'normal' sperm head further by means of scanning electron microscopy to detect any abnormality.

MATERIALS AND METHODS

The semen from 24 male partners of infertile couples, married for several years, with an ejaculate volume of at least 2 ml, a minimum sperm concentration of over 20 million per ml, and a motility percentage of 50 or more were studied. This semen was divided into two parts of 1 ml each. The sperm concentration, percentage of motile sperms, and morphology of the first part of the semen sample were evaluated using smear preparations stained for 5 minutes with 0.04% toluidine blue.[13] To the second sample, 1 ml of Tyrode solution was carefully added to separate the motile and nonmotile sperm by the swim up migration procedure.[14] After 1 hour of swim up, the Tyrode solution containing motile spermatozoa was separated for examination of sperm concentration, motility percentage and morphology by means of a swab preparation and observed under the light microscopy. In addition, swab preparations were made on cover glasses and coated with carbon and gold for examination under scanning electron microscope.[11]

RESULTS AND DISCUSSION

The results of this study on density, motility and velocity of the spermatozoa before and after swim up migration procedure and observed under the light microscopy are summarized in Tables 1 and 2. Sperm concentration before swim up migration was about 30 - 85 x 10^6 per ml, with a mean of 52.5 x 10^6 per ml. After migration, the concentration was about 1 - 12 x 10^6 per ml, with a mean of 4.6 x 10^6 per ml. There was a reduction of 8.6% after migration. In a similar study, Moeloek achieved a much lower result, 1.7 x 10^6 per ml or 3.4% of the original sample.[15]

Table 1. Results of spermatozoal parameters (mean values) before and after the swim up migration procedure

	Spermatozoa		
	Density (10^6/ml)	Motility (%)	Velocity (seconds)
Before swim up migration	52.5	61.5	44.4
After swim up migration	4.6	90.6	52.4

Although the resulting sperm concentration was very small, certain advantages was gained. Sperm motility was increased from 61.5% to 90.6%. Sperm velocity, a distance of 1/20 mm covered by the sperm per unit of time, was also increased from 44.4 to an average of 52.4 micrometer per second. These are qualities essential to artificial insemination and in vitro fertilization.

Table 2 shows that with swim up migration, there was an increase in the percentage of normal sperms from 48.5% to 60.9%, and a decrease in the percentage of abnormal sperms from 51.5% to 39.1%. With this procedure it was not possible to remove all abnormal sperms. This meant that any sperm with intact tails, even those with some form of head abnormality, would still be able to migrate to the top and be trapped in the Tyrode solution. Of the various forms of head abnormalities it was found that microcephalic sperms predominated, both before (20.2%) and after (18.2%) migration. Sperms with spheroid heads were usually without acrosomes and therefore were not capable of fertilizing the ovum. The morphology of the tapering and amorphous sperms was very far from normal, and consequently it can be predicted, that their DNA can

also be expected to be abnormal, although it was not proven in this investigation. With swim up migration procedure, it was clear that only the percentage of normal sperm will increase, while that of abnormal ones will decrease. This means that sperms with some form of abnormality will still be present, and will not necessarily lose in the competition to fertilize.

Table 2. Sperm head morphology in 24 samples, before and after swim up migration procedure

Morphology	Normal mean	Abnormal mean	Type of head abnormality mean						
			Mac	Mic	Sph	Tap	Pyr	Amo	Dou
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Before migration	48.5	51.5	4.6	20.2	3.4	6.1	8.6	8.0	0.6
After migration	60.9	39.1	3.2	18.4	1.2	4.0	5.9	6.4	0.1

Note: Mac = macrocephalic; Mic = microcephalic; Sph = spherical; Tap = tapering; Pyr = pyriform; Amo = amorphous; Dou = double heads

The pyriform sperm has a normal sized head, with the only difference being the pointed caudal end. Macrocephalic and pyriform sperms would probably be capable of fertilization, however it should be further proven by their fertilizing capacity. If both macrocephalic and pyriform sperms were included, sperm concentration was 13.2% before- and 9.1% after migration. If all 3 shapes (normal, macrocephalic and pyriform) were added up, sperm concentration was 61.7% before- and 70.0% after migration. Unfortunately at present, no techniques are available for excluding such abnormal forms (macrocephalic and pyriform from the normal head sperms). These 2 shapes were considered "normal" in their fertilizing capacity because they all possess normal sized acrosomes. It is important to determine whether acrosomal enzymes are present and whether these "normal" sperms are capable of penerating the hamster zona free oocyte.

For scanning electron microscopy, the specimens were coated with carbon and gold without prior staining. At a magnification of x 7,200 - 18,000, defects of the sperm head could be detected. With the scanning electron microscope defects of the head can be easily detected. These findings were similar to those of Hafez and Kanagawa.[11] The examination of spermatozoa by scanning electron microscopy may be useful in special cases, but is not recommended for routine use due to the high cost.

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